Correlation of Organic Cation/Carnitine Transporter 1 and Multidrug Resistance-Associated Protein 1 Transport Activities with Protein Expression Levels in Primary Cultured Human Tracheal, Bronchial, and Alveolar Epithelial Cells

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ABSTRACT: Understanding how transporters contribute to the distribution of inhaled drugs in the lung is important for the discovery and development of such drugs. Protein expression levels may be useful to predict and understand drug distribution. As previously reported, organic cation/carnitine transporter 1 (OCTN1) and multidrug resistance-associated protein 1 (MRP1) have higher levels of protein expression among transporters in primary cultured human lung cells. Nevertheless, it is unclear to what extent transport activity correlates with transporter protein expression. The purpose is to evaluate whether differences in OCTN1 and MRP1 protein expression govern the respective transport activity in primary cultured human lung cells. The model substrates of 4-[4-(dimethylamino) styryl]-N-methylpyridinium iodide (ASP^+) and carboxy-dichlorofluorescein (CDF) for OCTN1 and MRP1, respectively, were used in the lung cells from five donors. Significant correlation was found between the kinetic parameter V_{max} for ASP^+ and OCTN1 protein expression in plasma membrane of tracheal, bronchial, and alveolar cells (r^2 = 0.965, 0.834, and 0.877, respectively), and between the efflux of CDF and MRP1 protein expression in plasma membrane of tracheal, bronchial, and alveolar cells (r^2 = 0.800, 0.904, and 0.790, respectively). These findings suggest that OCTN1 and MRP1 protein concentrations are determinants for drug distribution in the lung. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

Inhalation is an attractive alternative route to oral and invasive routes for drug delivery for treating diseases of the lung or accessing the systemic circulation.[1] Therefore, understanding drug distribution in patient lungs is very important for the discovery and development of inhaled drugs as well as for the best treatment options for individual cases. Thus, studying freshly isolated biopsies from individual patients would be the most appropriate method for assessing drug distribution in the lung because the biological features of fresh biopsies are considered similar to the features of the intact human body. However, although the evaluation of gene and protein expressions could be technically possible through the use of fine needle aspiration biopsy, it still remains challenging to extract sufficient numbers of cells with high viability to measure the physiological functions of their target proteins. In contrast, primary cultured human lung cells are currently commercially available and are already in use for investigation of drug disposition. Although the differences between primary cultured cells and cells from freshly isolated biopsies remain unclear, primary cultured cells are a promising tool that enables us to evaluate transport activities.

The lung anatomically comprises the trachea, bronchi, and alveoli, and the cellular composition of the respiratory epithelium varies by region.[2] Furthermore, airway diseases are region specific with tracheomalacia and Mounier–Kuhn syndrome specific to the trachea, bronchitis, and asthma specific to the bronchi, and emphysema specific to the alveoli. Drug delivery to pulmonary regions may be affected by device, formulation, and inhaled particle size. Aside from these, the mechanisms of drug distribution in different pulmonary regions may be a decisive factor for the efficient medication of lung diseases after the inhaled drugs reach the target regions. It is widely accepted that drug transporters play an important role in the absorption and extrusion of drugs resulting in alterations in the drug concentration in target organs. However, as mentioned above, it is possible to quantify gene/protein expressions of transporters from a lung biopsy but not to measure their transporter function. Therefore, a method to predict transporter activity from their gene/protein expressions in a biopsy sample is of high priority.

Abbreviations used: ASP^+, 4-(4-(dimethylamino) styryl)-N-methylpyridinium iodide; CDF, carboxy-dichlorofluorescein; HPAEpiCs, human pulmonary alveolar epithelial cells; HTEpiCs, human tracheal epithelial cells; MRP, multidrug resistance-associated protein; NHBEs, normal human bronchial cells; OCTN, organic cation/carnitine transporter; SLC, solute carrier.

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Ohtsuki et al.[3] determined the protein expression profile of drug transporters in the plasma membrane fraction and metabolizing enzymes in the microsomal fraction of 17 human liver biopsies. They demonstrated that the activity of cytochrome P450 enzymes was better correlated with enzyme protein expressions than with mRNA expression in the microsomal fraction. There are several correlative studies reported between protein expression and their transport functions, for example, glucose transporter 1 in human brain capillary endothelia[4] and serotonin transporter in fibromyalgia[5]; however, these studies used relative indicators such as maximal membrane binding capacity (B_{max}). In contrast, Shirasaka et al.[6] showed the fundamental parameters, such as K_m and V_{max}, for P-gp-mediated transport were well correlated with expression levels of P-gp using Western blot analysis. Ligand-binding assays such as ELISA and Western blot were widely used; nevertheless, the specificity of the antibody was sometimes problematic. The quantitative targeted absolute proteomics by liquid chromatography–mass spectrometry (LC–MS/MS) was one of the useful approaches to generate more accurate quantitative values of protein expression. To date, there have been no reports showing the correlation of transport activities with protein expression by LC–MS/MS-based quantification; thus, this approach would be worthwhile to evaluate such correlation more precisely.

Recent LC–MS/MS-based quantification has demonstrated that there are interindividual differences in transporter protein expression. For instance, there was an 8.38-fold maximal interindividual difference in MDR3 protein expression in the plasma membrane fraction of the liver from 17 donors,[3] and there was a 4.24-fold maximal interindividual difference in EAAT1 expression in the whole tissue lysate of the blood–brain barrier from seven donors.[7] A significant 18.0-fold interindividual difference in multidrug resistance-associated protein (MRP1) protein expression was found in the plasma membrane fraction from the bronchial region; however, organic cation/carnitine transporter 1 (OCTN1) protein expression in the plasma membrane fraction from the tracheal region demonstrated at most a 2.79-fold interindividual difference.[8] The previous data showed that 16 of 18 transporters had higher expressions in the plasma membrane of the female lung than that in the male lung tissues, especially MRP8 had a 7.32-fold maximal gender difference,[8] whereas there have been no reports of age and ethnicity differences of protein expressions for the transporters in the human lung. Furthermore, MRP1 expression in bronchial biopsies of chronic obstructive pulmonary disease (COPD) patients was lower than those of healthy controls,[9] and MRP1 activity is affected by cigarette smoke extract, suggesting that smoke could decrease MRP1 protein expression.[10] Therefore, determining interindividual differences in transport activity and in the relationship between transport activity and protein expression in the plasma membrane fraction are crucial for understanding drug distribution in the lungs of different individuals.

The purpose of this study was to evaluate whether differences in OCTN1 and MRP1 protein expression in the plasma membrane fraction govern the respective transport activity by examining the correlation between protein expression and transport activity in primary cultured human lung cells that were derived from the tracheal, bronchial, and alveolar regions of five individual donors.

MATERIALS AND METHODS

Materials

Primary cultured normal human bronchial cells (NHBEs) were purchased from Lonza (Basel, Switzerland). Primary cultured human tracheal epithelial cells (HTEpiCs) and human pulmonary alveolar epithelial cells (HPAEpiCs) were purchased from ScienCell (Carlsbad, California). Cells from the five donors were seeded in 24-well plates (Corning, Amsterdam, The Netherlands) at a density of 35,000 cells/cm^2 and cultured in bronchial epithelial cell growth medium (NHBEs), bronchial epithelial cell medium (HTEpiCs), or alveolar epithelial cell medium (HPAEpiCs) for 2 days at 37°C in 5% CO_2, after a single passage at the manufacturers. All donors used in this study were the same as those used in a previous study that quantified protein expression of drug transporters in the lungs.[8] The research protocols for this study were approved by the Ethics Committees of Nippon Boehringer Ingelheim Company, Ltd. and the Graduate School of Pharmaceutical Sciences, Tohoku University, and all procedures, including purchase of cells, sample preparation, and all experimental works, were performed at Nippon Boehringer Ingelheim Company, Ltd.

Evaluation of OCTN1 Uptake Transport Activity

Uptake of the cationic fluorescent substrate of OCTN1, 4-(4-(dimethylamino) styryl)-N-methylpyridinium iodide (ASP^+), was investigated at different concentrations. All experiments were performed in bicarbonated Krebs–Ringer buffer (KRB) that is composed of 15 mM HEPES, 116.4 mM NaCl, 5.4 mM KCl, 0.78 mM NaH_2PO_4, 25 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 0.81 mM $MgSO_4$, and 5.55 mM glucose at pH 7.4, which was previously used for the ASP^+ uptake study.[11] The concentration dependence of ASP^+ uptake was studied by incubating each cell individually with ASP^+ (at 10, 50, 100, 200, 500, and 1000 μM final concentrations) for 20 min at 4°C or 37°C. Cells were rinsed twice with ice-cold KRB and then lysed with 1% (w/v) Triton X-100. The fluorescence of samples was measured with an automated plate reader (FLUOstar Galaxy, BMG Labtech, Offenburg, Germany) at excitation and emission wavelengths of 485 and 590 nm, respectively. All uptake studies were performed in triplicate for each donor. The average values were calculated from a triplicate assay, and data represent mean of average values from five donors.

Evaluation of MRP1 Efflux Transport Activity

Efflux of the MRP1 probe, carboxy-dichlorofluorescein (CDF), was investigated to evaluate MRP1 activity. All experiments were performed in modified Tris/sucrose buffer for efflux transport study,[12] including 50 mM Tris–HCl, 250 mM sucrose buffer, pH 7.4 (Tris/sucrose buffer), and containing 20 mM $MgCl_2$ and 4 mM phosphocreatine. Each cell was preloaded with 5 μM CDF for 2 h at 37°C, rinsed twice with ice-cold phosphate-buffered saline (PBS), and lysed with 1% (w/v) Triton X-100 (control, −MK571, 0 h). To evaluate the release of CDF from each cell, cells were incubated for 1 or 3 h either with or without MK571 (20 μg/mL), a model inhibitor of MRP1. Cells were then rinsed twice with ice-cold PBS and lysed with 1% (w/v) Triton X-100. The fluorescence of samples was measured with an automated plate reader (FLUOstar Galaxy) at excitation and emission wavelengths of 485 and 520 nm, respectively. All

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